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IMPACT ON THE QUALITY CHARACTERISTICS OF ICE CREAM BY USING POLYMERIZED WHEY PROTEIN ISOLATES

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Various type of stabilizers are being use in food industry among them whey proteins are promising stabilizers widely used as a functional and nutritional component in dairy products. These have many functional properties *i.e* viscosity, foaming ability, water holding capacity, solubility, gelation and emulsification. This study was conducted to evaluate the impact of polymerized whey protein isolates (PWPI) as substitute for stabilizer and emulsifier on the quality parameters of ice-cream. The effects on different quality parameters were determined and compared to control with 0.5% emulsifier (0.2% lecithin) and stabilizer (0.3% guar gum). Compositional analysis (moisture, ash, fat, lactose and nitrogenous fractions), Physiochemical analysis (pH and acidity), Textural analysis (viscosity, firmness, overrun and meltdown rate) were done. It was observed that there was slight change in pH and acidity whereas significant increase was observed for viscosity, overrun and structural retention by increasing PWPI concentration. Firmness and meltdown behavior were decreased by increasing PWPI contents as compared to the control. Conclusively, addition of PWPI as protein based emulsifier and stabilizer revealed additional functional properties in ice-cream.

Keywords: Whey protein isolates, polymerization, ice cream, functional ingredient, quality attributes.

INTRODUCTION

Ice cream is popular dairy product, in wider sense it covers a range of different frozen desserts including dairy ice cream, non-dairy ice cream, gelato, frozen yoghurt, milk ice, water ice and fruit ice (Clarke, 2004). Its local definition is a pure clean frozen product made from combination of milk and cream, with or without eggs, with potable water, sugar, harmless flavors and harmless colors (The Punjab Pure Food Rules, 2007). Ice cream dairy based dessert and one of the main products in the dairy industry and it has become a leading consumer product for a large number of the population (Warke *et al.,* 2000). Ice cream ingredients during processing produce microstructure consist of air bubbles, fat droplets in a size of 0.1 to 1µm, ice crystals and solution of sugar, milk proteins and polysaccharides called as matrix (Innocente, 2002). Proteins act as surface active agents help to stabilize texture by absorbing water and establish gas-liquid interfaces and also stabilize the bubbles (Clarke, 2004). High fat content made firm body due to frozen fat particles that become firm while freezing but too much fat cause hard texture of ice cream (Dubey and White, 1997).

Whey proteins are the co-product of cheese and caseinates manufacturing industry (Kinsella and Whitehead, 1989). It contains a high content of proteins with wider range of functional and nutritional properties. Different food functional properties include solubility, heat stability, dispersibility, gel network formation, edible films, and surface activity (Foegeding *et al.,* 2011). Hence whey proteins as ingredient have a broader range of functional applications in food industries. These functional properties involve gelation, foam formation, thermal stability and emulsification (Foegeding *et al.,* 2002; Graham *et al.,* 1981). Whey is basically cheese industry product. Whey volume is increasing in the world due to high production of cheese. The annual produced volume of cheese is 100×10^9 L. This huge volume relates to a bulk of protein source which is utilized by the industry of about 500×10^6 Kg annually (McIntosh *et al.,* 1998). (Patel, 2006). Similarly increase amount of whey protein lower the surface tension and increase the viscosity (Ruger, 2001). Whey proteins have become a component of many commercially available ingredient blends which contribute towards the texture, flavor and nutrition of a broader range of dairy as well as other food products (Solorio *et al.,* 2011). Whey protein contains high amount of essential amino acids mainly the branched chain amino acids have made whey protein an important nutritional ingredient. (Burrington and Agarwal, 2012).

Polymerized whey proteins are made by heating whey protein isolate (WPI) at neutral pH and different salt concentrations for gelation (Wit, 1998). These gels have high water holding capacity, more fracture stress and fracture modulus and the

translucency (Vardhanabhuti *et al.,* 2001). Whey protein modifications involve enzymatic hydrolysis or heat-induced polymerization. These modifications change the gelation and interfacial properties of whey proteins (Foegeding *et al.,* 2002). Gelation of globular proteins through heat is completed in two separate stages. First is partial unfolding i-e denaturation of the globular protein structure and second is intermolecular aggregation of denatured protein (Bolliger *et al.,* 2000) In denaturation intra-molecular bonds are broken down *i.e* non-covalent and disulfide bonds. In aggregation step, new bonds are formed between protein molecules (Totosaus *et al.,* 2002; Fitzsimons *et al.,* 2007). *β*-lactoglobulin is considered as the major protein constituent which cause aggregation of WPI on heating while a-lactalbumin presence cause differences in the aggregation behavior (Kazmierski *et al.,* 2003). This study was conducted to polymerize the WPI to incorporate PWPI into the ice cream in various concentrations of 3%, 5% and 7%, to substitute the stabilizer and emulsifier with various PWPI concentrations in ice cream mix and finally determine the impact of PWPI on quality attributes of ice cream such Compositional analysis (moisture, ash, fat, lactose and nitrogenous fractions), Physiochemical analysis (pH and acidity), Textural analysis (viscosity, firmness, overrun and meltdown rate) and at the end sensory evaluation of the product was also analyzed.

MATERIALS AND METHODS

***Materials*:** Buffalo milk was purchased from the dairy herd of Livestock Department, University of Agriculture Faisalabad. Physicochemical analysis of purchased milk, native whey protein and PWPI were done by AOAC 2003 method. Milk fat was purchased from the local dairy sale point. Emulsifier, stabilizer, flavor, sugar and whey protein isolates were purchased from the Gool, Mintgamri Bazar Faisalabad.

Table 1: Treatment Plan

Ingredient	T_0	T_1	T_2	T_3
Sugar (g)	75.0	75.0	75.0	75.0
Stabilizer (guar gum)	0.3%	-	-	-
Emulsifier (lecithin)	0.2%	-	-	-
Cream (34 % fat) ml	78.5	73.5	73.5	71.5
Milk (7% fat) ml	344.0	334.5	326.5	318.5
PWPI (g)	-	15.0	25.0	35.0

***Preparation of polymerized whey proteins*:** 10% solution of whey protein isolate was prepared after that 0.1 M NaOH was added to adjust the pH of solution at 7 when required pH was obtained then solution was heated at 80^0 C for 30 minutes after that polymerized whey protein was obtained which was gel like then was stored at room temperature (Eissa, 2013). Then it was used as a substitute of emulsifier and stabilizer in ice cream.

***Preparation of ice cream*:** Ice cream was prepared in small electric machine having 2Kg capacity with 60 rpm and hardening is done in freezer. Recipe used were 15% sugar, 12% fat, 0.3% stabilizer, 0.2% emulsifier and vanilla flavor. The PWPI was incorporated into vanilla ice cream in three treatments of 3%, 5% and 7% and compared with the control having 0.5% emulsifier (0.2% lecithin) and stabilizer (0.3% guar gum) jointly.

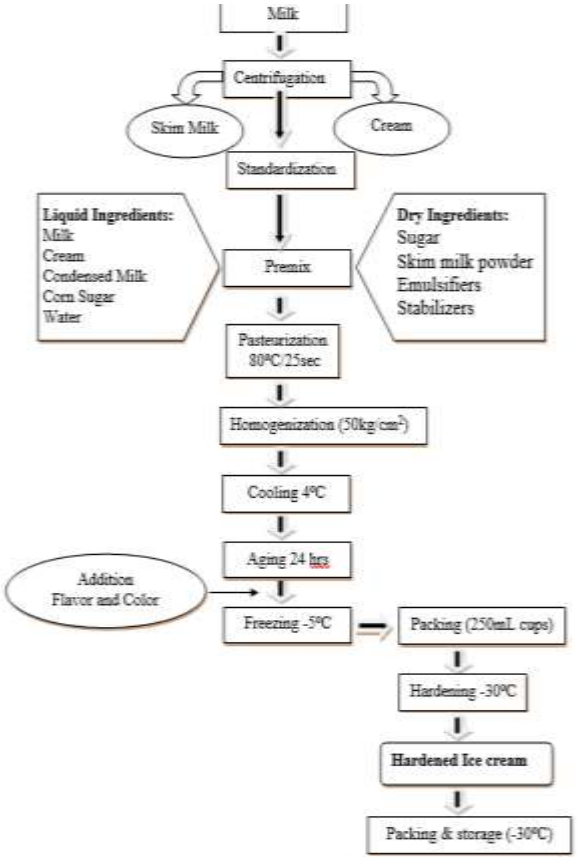

Fig. 1. Flow diagram of Ice cream formation

***Chemical analysis of ice cream*:** Ice cream sample were analyzed for compositional analysis (moisture, ash, fat, lactose and nitrogenous fractions), Physiochemical analysis (pH and acidity), According to AOAC 2003 method and Sensory evaluation (surface appearance, texture, color, taste, mouth feel and ice crystal perception, palatability and overall acceptability).

***Viscosity*:** Viscosity of the ice cream samples was measured after thawing at refrigeration temperature using viscometer Brookfield LVDVE-230 (MA, USA) by using spindle number 4 after every 15sec reading was noted (Sevim and Takin, 2001).

***Firmness*:** The ice cream was added to a 33mL cylindrical container and kept -17± 2ºC for 48 hours. The measurements were obtained at room temperature (25ºC ± 2ºC), using a

texture analyzer (TA-XT2i, Stable Microsystems Ltd., UK) equipped with a 5-mm stainless steel cylindrical probe. The penetration speed of the probe was 2.0mm/s to a distance of 5, mm Test speed 3mm/s; post-test speed 10mm/s, data acquisition rate 250pps, trigger type auto 20g (Aime *et al.,* 2001)

***Overrun*:** Overrun of the product was determined as per AOAC (2003). Overrun was estimated using a standard 1000mL beaker, according to the equation as follow

$$\% \text{ Overrun} = \frac{\text{Volume of Ice cream} - \text{Volume of mix}}{\text{Volume of mix}} \times 100$$

***Melt Down*:** Ice cream samples (100g) were removed from the containers, put on a wire screen with 2 mm openings supported by a funnel on a ring stand with a 100mL graduated cylinder underneath. The sample and test assembly were placed immediately at 25°C, and the volume of serum collected was measured for a period of 30 min, when total volume of serum and serum weight were recorded (Cody *et al*., 2007; Lee and White, 1991). Melting rate was calculated as follows.

$$\% \text{ Melting} = \frac{\text{weight of serum}}{\text{weight of ice cream}} \times 100$$

***Statistical analysis*:** Results were analyzed by using Completely Randomized Design and ANOVA.

RESULTS AND DISCUSSION

***Compositional analysis of native and pwpi*:** Native whey protein isolates (WPI) had 92.5% protein, 2.93% ash and 6.55% moisture while 10% w/v PWPI had 8.7% protein, 0.641 ash, 82.8% moisture and viscosity mean value was 10925.

Table 2. Compositional analysis of buffalo raw milk

Compositional Parameter	% Value
Moisture	82.00
Fat	7.00
Protein	4.40
Lactose	6.10
Ash	0.80
Total solids	18.30

Table 3: Compositional analysis of Treatments

Sample	Ash	Fat	Lactose	T.N
T_0	0.32^a	11.8^a	4.20^b	4.31^c
T_1	0.23^b	11.9^a	4.63^a	4.82^b
T_2	0.25^b	11.9^a	3.79^c	5.66^a
T_3	0.34^a	11.9^a	3.56^d	5.93^a

***Compositional analysis of ice cream*:** Fat content has major importance in ice cream. It acts as a carrier of flavor and contributes to its own flavor (Frost *et al.,* 2005). Good percentage use is essential to had better quality of ice cream. Fat content do not had impact on freezing point but it retards the whipping. So, fat content plays key role in development of physical characteristics like flavor, texture, mouth feel and appearance (Potter and Hotchkiss, 1995). The fat content of ice cream samples has decreasing trend with the increase in PWPI concentrations. Decreasing trend was higher in T_0 with reference to T_1 and T_2 with reference to T_3. Control sample had highest fat content whereas the fat content of T_1 was close to control. Fat percentage was adjusted, as prescribed in the recipe up to 12% but increasing PWPI concentrations from 3 to 5% results in decrease in fat and milk ratio. So PWPI concentrations had significant effect on the percentage fat content of ice cream.

The proteins are polymers of amino acids and their amount in a sample represents its quality index. The crude protein is normally determined by measuring the amount of nitrogen in a sample. Total nitrogen contributes to the flavor, body and texture to the finish product. It is also essential for the formation of small stable air cells.

The total nitrogen content of ice cream samples increases as a function of increase of PWPI concentrations. Increasing trend was higher in T3 compared to T0 and T2 with reference to T1. Control sample had lowest total nitrogen content whereas the total nitrogen content of T1 was close but higher to control. T0, T1, T2 and T3 treatments mean values are 4.31%, 4.83%, 5.66% and 5.93% respectively.

It was observed that the lactose content of ice cream samples decreases as a function of increase of PWPI concentrations. Decreasing trend was lowest in T_3. T0 had highest lactose content. T0, T1, T2 and T3 treatments mean values are 4.550%, 4.416%, 3.796% and 3.566% respectively. The ash content is an inorganic residue remaining after the removal of water and organic matter by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals in a food. The ash content of ice cream samples increases as a function of increase of PWPI concentrations. Increasing trend was higher in T3 with reference to T2 and T2 with reference to T1. T3 had highest percent ash whereas control had the lowest. T0, T1, T2 and T3 treatments mean values are 0.210%, 0.233%, 0.253% and 0.340%, respectively.

Moisture, ash and total nitrogen content showed increasing trend with the PWPI concentration while lactose showed significantly decreasing trend and fat content had no significant change.

***Physicochemical analysis of ice cream*:** pH and Acidity was determined in physicochemical analysis of ice cream treatments. There was not much difference between the treatments for the pH and acidity.

The pH of control sample was slightly lower than the other treatments. PWPI initial pH was adjusted to neutrality prior to incorporate into ice cream sample so all the PWPI treatments had same pH value as compared to T0. T0, T1, T2 and T3 treatments mean values are 6.726, 6.733, 6.734 and 6.734 respectively. According to Kanbakna *et al.,* (2004) good ice cream has pH near neutrality and in the range of 6-7.

The acidity of ice cream samples slightly decrease as a function of incorporating PWPI as compared to control. PWPI initial pH was adjusted to neutrality prior to incorporate into ice cream samples, so all the PWPI treatments had same acidity values while T0 had the highest acidity value. T0, T1, T2 and T3 treatments mean values are 0.192%, 0.183%, 0.179% and 0.183% respectively as denoted in Table 3.

Table 3. Comparison of different treatments for physico-chemical analysis

Sample	pH	Acidity
T_0	6.76^a	0.16^b
T_1	6.73^b	0.18^a
T_2	6.73^b	0.17^a
T_3	6.73^b	0.18^a

***Textural analysis*:** Viscosity is normally defined as resistance to flow and unit of its measurement is centipoise (cP). Viscosity can be affected by type of food, temperature and fat globules size and distribution. Hydration of protein tends to increase the viscosity in ice cream. Air incorporation, creaming, flow rate, flow conditions and casein miceller aggregation can be determined by the viscosity (Ye, 2008). The viscosity of ice cream samples increases as a function of increase of PWPI concentrations. Increasing trend was higher in T3 with reference to T2 and T2 with reference to T1. Control sample had lowest viscosity whereas the viscosity of T1 was close but higher to control. T0, T1, T2 and T3 treatments mean values are 980.00cP, 991.67cP, 1328.3cP and 1660.0cP respectively. Ice cream firmness relates to its structure, air cells distribution, fat and ice crystals presence in the structure (Aime et al., 2001). Ice cream texture become softer and firmness decreases with the increase of protein contents in ice cream (Young, 2007). The firmness of ice cream samples decreases as a function of increase of PWPI concentrations. Decreasing trend was higher in T3 with reference to T2 and T2 with reference to T1. Control sample had highest firmness whereas the firmness of T1 was close to control. T0, T1, T2 and T3 treatments mean values are 21.23g, 20.06g, 19.13g and 17.45g respectively. Melt down was also lowered by the increment of whey protein concentration as minimum was observed in $T_{3\ while}$ over run was observed to be increased with the increasing concentration of whey protein as described in Table 4.

Table 4. Mean values comparison of different treatments for textural analysis

Sample	Viscosity	Firmness	Overrun	Melt down
T_0	980^c	21^d	41^c	37^{ab}
T_1	991^c	20^c	38^c	41^a
T_2	1328^b	19^b	47^b	36^b
T_3	1660^a	17^a	55^a	28^c

***Sensory evaluation*:** Sensory evaluation by the judges has different preference.

The mean score for surface appearance of ice cream samples increases as a function of increase of PWPI concentrations up to 5%. Increasing trend was higher in T_2 with reference to T_1 and T_1 with reference to T_0. T_3 had lowest surface appearance scores whereas the surface appearance of T_1 was close to control. T_0, T_1, T_2 and T_3 treatments mean scores are 6.63, 6.79, 7.64 and 6.44 respectively.

Sensory results for texture analysis of ice cream samples were nonsignificant with respect to PWPI concentrations. Texture of the treatments scores were close to each other while T_2 had maximum scores. T_2 has good texture by the judge's point of view as compared to control, so 5% PWPI concentration is better to had good ice cream texture as well as a good replacer of emulsifier and stabilizer.

Color is the perception of the product which attracts the consumers. It is the most important quality parameter of the product because if the color of the product is not accordance to the ingredients used and the description of the product than the product will lose its acceptability.

It was observed that T2 sample has the most appealing color as compared to other treatments.

It is the most important organoleptic property of any food product, which defines the acceptability of the product. Taste is the perception of the consumer either the product is sweet, salt, sour, chalky etc.

The mean score for taste of ice cream samples increases up to 5% PWPI concentration and then decreases as a function of increase of PWPI concentrations. Increasing trend was higher in T_2 with reference to T_1 and T_1 with reference to T_3. T_0 had lowest taste mean scores whereas the taste of T_3 was close to control. T_0, T_1, T_2 and T_3 treatments mean scores are 6.00, 6.97, 7.78 and 6.34 respectively.

The mean score for mouth feel of control ice cream was higher than samples with PWPI. Scoring trend was higher in

Table 5: Sensory Evaluation of Ice Cream.

Sample	Surface	Texture	Color	Mouth feel	Ice crystals	Palatability	Overall Acceptability
T_0	6.6^b	6.5^a	6.5^a	7.4^{ab}	7.3^a	7.7^a	7.3^{ab}
T_1	6.7^{ab}	6.5^a	6.1^a	6.6^b	6.2^b	6.5^b	6.8^{bc}
T_2	7.6^a	7.2^a	7.3^a	7.5^a	7.1^a	7.5^a	7.7^a
T_3	6.4^b	6.2^a	6.5^a	6.4^c	6.2^b	6.6^b	6.5^c

T_0 with reference to T_2 and T_2 with reference to T_1 and T_1 with reference to T_3. T_1 and T_3 had lowest mouth feel mean scores whereas the mouth feel of T_2 was close to control. T_0, T_1, T_2 and T_3 treatments mean scores are 7.40, 6.67, 7.57 and 6.50 respectively.

Ice crystals are formed due to the defective freezing and hardening processes, low quality fat used in the product recipe, and lactose and other sugar products of low quality. The formation of the ice crystals also indicates the presence of high amounts of the sugar and low level of fat. These ice crystals will form sandiness in the ice cream.

The mean score for ice crystals perception of control ice cream was higher than samples with PWPI. Scoring trend was higher in T_0 with reference to T_2 and T_2 with reference to T_3 and T_3 with reference to T_1. T_1 had lowest ice crystals perception mean scores whereas the ice crystals perception of T_2 was close to control. T_0, T_1, T_2 and T_3 treatments mean scores are 7.34, 6.23, 7.16 and 6.29, respectively.

Palatability of ice cream is generally effected by its overrun, more overrun leads to less palatability. Palatability of ice cream is generally effected by its overrun, more overrun leads to less palatability.

The mean score for palatability of control ice cream was higher than samples with PWPI. Scoring trend was higher in T_0 with reference to T_2 and T_2 with reference to T_3 and T_3 with reference to T_1. T_1 had lowest palatability mean scores whereas the palatability of T_2 was close to control. T_0, T_1, T_2 and T_3 treatments mean scores are 7.76, 6.51, 7.57 and 6.62 respectively.

T_2 were considered excellent in various parameters followed by T_0 and T_1 have poor acceptability while T_3 was considered to have less desired features. Surface appearance, texture, color, taste and flavor was good for T_2, sweetness level get equal scores by judges as pre-standardized in recipe. Palatability mean scores were high for T_0. Mouth feel was good as compared to control. Whereas much ice crystals perception scores were for T_1 and T_3 and T_0 have the best results and T_2 resembles to control. Overall acceptability for T_2 with 5% PWPI was considered good by judges.

***Conclusion*:** PWPI acts as functional ingredient contributes to various beneficial properties in ice cream most important with increase in overrun, viscosity decreasing melt down and firmness and several good sensory attributes that renders it fit for use as a functional ingredient in ice cream. Higher concentrations contribute to fluffy texture and ice crystals formation. So PWPI concentration up to 5% is fit to obtain better quality.

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